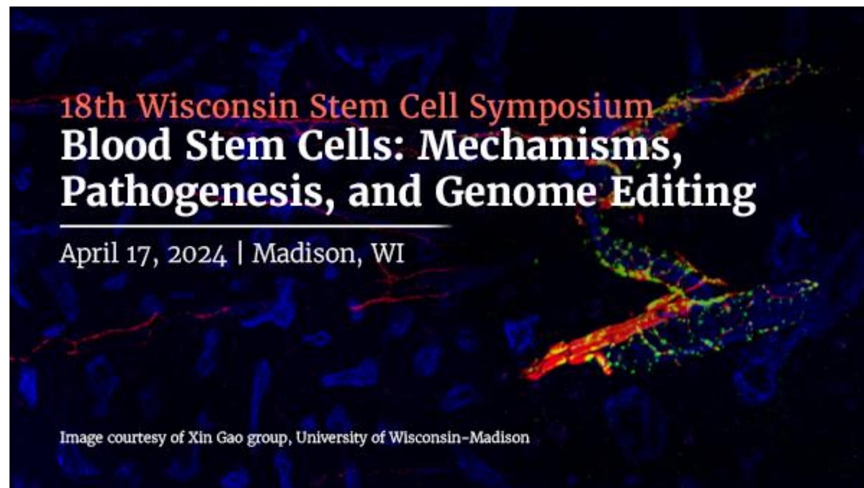

NOTE: Each submitter's name is in bold and italicized.

POSTER CONTEST FINALISTS

1. A gain-of-function p53 mutant synergies with oncogenic NRAS to upregulate KLF4 and promote acute myeloid leukemia

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Acute myeloid leukemia (AML) is an aggressive blood cancer that predominantly occurs in the aged population. We have previously demonstrated that a subset of AML patients with concurrent RAS pathway and TP53 mutations have an extremely poor prognosis and that most of these TP53 mutations are missense mutations. In contrast to the mixed AML and T cell malignancy that developed in NrasG12D/+ p53–/– (NP–/–) mice, NrasG12D/+ p53R172H/+ (NPmut) mice rapidly developed acute inflammation-associated AML. Under the inflammatory conditions, NPmut hematopoietic stem and progenitor cells (HSPCs) displayed imbalanced myelopoiesis and lymphopoiesis and largely normal cell proliferation despite MEK/ERK hyperactivation. RNA-Seq analysis revealed that oncogenic NRAS signaling and mutant p53 synergized to establish an NPmut-AML transcriptome distinct from that of NP-/- cells. The NPmut-AML transcriptome showed elevated expression of inflammatory genes, including those linked to NFkB signaling. NFkB was also upregulated in human NRAS;TP53 AML. Our data suggests that mutant p53 may interact with a transcription factor (TF) downstream of RAS/MEK/ERK to promote acute inflammation and AML. We identified KLF4, a master TF that regulates cell proliferation and apoptosis under inflammation, as the top upregulated TF in NPmut vs control HSPCs. Reciprocal IP-Western blot analysis

revealed that endogenous KLF4 indeed forms a complex with endogenous mutant p53 in human NPmut AML KY821 cells. Kenpaullone, a small molecule that can replace KLF4 in the reprogramming of fibroblasts to pluripotent stem cells, induced KLF4 upregulation and increased growth and colony formation in KY821 cells. By contrast, knocking down KLF4 downregulated the expression of inflammatory genes and significantly inhibited KY821 cell growth in vitro. Using a genetic approach, we showed that downregulation of KLF4 decreased the expression of inflammation-related genes, restored imbalanced myelopoiesis and lymphopoiesis, slowed down NPmut-AML progression, and prolonged the survival of NPmut mice. Our data suggests that mutant p53 interacts with KLF4 to promote acute inflammation and NPmut AML.

2. Defining gene regulatory network dynamics of leukemic stem cells in onset and relapse of T-ALL with integrative tri-modal sequencing and network inference

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Early studies suggest that the onset, progression, and relapse of T-cell acute lymphoblastic leukemia (T-ALL) is driven by a rare cell type called leukemic stem cells (LSC). Although these cells have been identified, the molecular signatures of LSC remain poorly characterized. To overcome this challenge, we have applied the tri-modal sequencing approach, TEA-seq, to measure the single-cell transcriptome, epigenome, and surface proteome of relapsing T-ALL. We have identified LSC's from the bone marrow (BM) biopsy of a T-ALL patient at the initial time of diagnosis as well as in a peripheral blood (PB) sample upon the patient's relapse. We applied cell-lineage tree-based gene regulatory network inference algorithm, single-cell Multi-Task Network Inference (scMTNI), which leverages the epigenetic and transcriptomic data from TEA-seq to determine cell-state-specific gene regulatory network (GRN). We have identified cell-state-specific GRNs for LSC collected from the BM and PB samples. We have identified a shared component of these networks which is driven by a common set of transcription factors including several factors, which regulate multiple target genes related to a leukemic stem-like cellular state. We have also identified unique network components of the BM sample indicative of the cellular state during early disease progression. Finally, we have identified regulatory programs specific to the PB-LSC which characterize the cell-state during early remission. In summary, our present study leverages tri-modal sequencing data as an input for scMTNI to identify and characterize the GRNs of LSCs as they transition through stages of leukemic disease progression and relapse.

3. Macrophages from Human Pluripotent Stem Cells

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Hematopoietic stem cells (HSCs) derived from the aorta-gonad-mesonephros (AGM) region hold significant promise for treating blood disorders and cancers. However, achieving their robust generation in laboratory settings remains a challenge. In this study, we successfully generated AGM-like hematopoietic progenitors from human pluripotent stem cells (hPSCs) by selectively inhibiting TGFβ during the developmental phase of aorta-like SOX17+CD235ahemogenic endothelium. These generated cells closely mimic primary cord blood HSCs at the molecular level, demonstrating both lymphoid and myeloid potential in in vitro cultures. Importantly, they exhibit effective homing to hematopoietic sites in zebrafish and demonstratethe ability to restore blood supply in bloodless zebrafish post-transplantation. Furthermore, these cells display engraftment and multilineage repopulating capabilities when transplanted into mouse recipients. Analysis through single-cell RNA sequencing unveiled a diverse array of lineage-primed progenitor populations. Utilizing their myeloid potential, we differentiated hPSCs into monocyte/macrophages, which are increasingly recognized as promising candidates for targeting solid tumors due to their natural ability to infiltrate tumors and their abundance within the tumor microenvironment. To evaluate their efficacy in targeting cancer cells, we employed CRISPR/Cas9 genome editing to introduce a chimeric antigen receptor CAR) into hPSCs, with a specific focus on targeting glioblastoma. The resulting CAR macrophages exhibited enhanced cytotoxicity against glioblastoma cells in vitro, offering a potential solution to the limited effectiveness of CAR T cell therapy in treating solid malignancies. Overall, our findings introduce a feeder-free culture system for generating AGMlike hematopoietic progenitor cells and subsequent immune cell production, presenting promising avenues for therapeutic interventions.

4. DDX41-dependent RNA Splicing Reveals Hematopoietic Mechanisms that are Dysregulated by Human DDX41 Clinical Variants

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Heterozygous DDX41 germline mutations occur in familial myelodysplastic syndrome/acute myeloid leukemia, and acute erythroid leukemia. DDX41 encodes an RNA helicase regulating RNA splicing, cGAS-Sting signaling, genome stability, and is required for murine hematopoietic stem/progenitor cell survival and differentiation1. We innovated a genetic rescue system to compare RNA-regulatory activities of wild-type and disease-associated DDX41 variants in HoxB8-immortalized murine myeloid progenitors2. To develop a broader perspective into lineage-specific/pan-lineage DDX41 activities, we extended this strategy to G1E-ER-GATA1 (G1E) erythroblasts. Total RNA from DDX41- or variant-expressing G1E-Ddx41+/-cells was analyzed by RNA-seq. Differential expression analysis revealed 138 Ddx41-regulated transcripts ($P < 0.05$), with 63 elevated and 75 decreased. We compared myeloid and erythroid DDX41-regulated transcripts to identify common and cell type-specific DDX41-regulated transcripts, which revealed 11 DDX41-regulated transcripts common to both systems.

To ask how broadly DDX41 regulates RNA processing, we conducted differential splicing analysis. In the myeloid cells, DDX41 increased 911 splicing events. G173R and R525H variants decreased

the events by 89% and 42%. Biological process analysis revealed variants retain activity to regulate transcripts associated with cell death and protein modification (p= 0.014, 0.04) but not those related to RNA metabolism and gene expression (p = 5.90E-09 for DDX41). In erythroid cells, DDX41 induced 1,438 splicing events, with 95% and 93% of the events not detected in cells expressing G173R and K331del variants. Biological process analysis demonstrated that variants fail to regulate transcripts related to RNA splicing, regulation of transcription, and chromatin remodeling (p=0.000009, 0.00009, 0.0002 for DDX41).

These studies established an innovative system to discriminate pathogenic from benign DDX41 variants and elucidate mechanisms that may lead to new hematopoiesis paradigms. By incorporating an ensemble of activity metrics and computational analysis, we are transforming the system to ascribe whether unknown variants closely resemble DDX41 or pathogenic variants to permit high-fidelity clinical curation.

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5. Pathogenic GATA2 Genetic Variants Utilize an Obligate Enhancer Mechanism to Distort a Multi-lineage Differentiation Program

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Mutations in genes encoding transcription factors inactivate or generate ectopic activities to instigate pathogenesis. By disrupting hematopoietic stem/progenitor cells, GATA2 germline variants create a bone marrow failure and leukemia predisposition, GATA2 Deficiency Syndrome, yet mechanisms underlying the complex phenotypic constellation are unresolved. We used a GATA2-deficient progenitor rescue system to analyze how genetic variation influences GATA2 functions. Pathogenic variants impaired, without abrogating, GATA2-dependent transcriptional regulation. Variants promoted eosinophil and repressed monocytic differentiation without regulating mast cell and erythroid differentiation. Using RNA-Seq, we discovered that human pathogenic variants (T354M and R307W) retain activity to regulate a cohort of GATA2 target genes; the variants were defective in regulating other GATA2 target genes. Furthermore, the variants also regulated ectopic target genes which were not GATA2-regulated. T354M occupied WGATAR-containing chromatin near its target genes, and deletion of these chromatin sites abrogated T354M-mediated gene regulation. While GATA2 and T354M required the DNA-binding C-terminal zinc finger, T354M disproportionately required the N-terminal finger and N-terminus for its function. GATA2 and T354M activated a CCAAT/Enhancer Binding Protein-��(C/EBP�) enhancer, creating a feedforward loop operating with the T-cell Acute Lymphocyte Leukemia-1 (TAL1) transcription factor. Elevating C/EBP� partially normalized hematopoietic defects of GATA2-deficient progenitors. Thus, pathogenic germline variation

discriminatively spares or compromises transcription factor attributes, and retaining an obligate enhancer mechanism distorts a multi-lineage differentiation program.

6. Genome Editing of Human Leukocyte Antigen Genes in Induced Pluripotent Stem Cells for Immune-Compatible Dendritic Cell Production: A CRISPR Study

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The success of regenerative medicine using allogeneic induced pluripotent stem cells (iPSCs) hinges upon precise genome editing of human leukocyte antigen (HLA) genes to mitigate the risk of immune rejection. Despite iPSCs' promise in regenerative medicine, HLA mismatch-induced immune rejection poses a significant challenge. This study aimed to generate dendritic cells from iPSCs with tailored HLA expression profiles to circumvent alloreactivity with the goal to engineer iPSCs to retain a specific class I HLA allele (HLA-A02) capable of activating T cells from about 40% of donors, while eliminating other less common HLA molecules likely to trigger alloreactivity. We also wanted HLA-C expression to be retained to confer protection against natural killer cells. We designed several gRNAs to allow for the selective editing of HLA- and HLA-B alleles using CRISPR/Cas9 in iPSCs with HLA-A02, HLA-A32, HLA-B44, HLA-B51, HLA-C3 and HLA-C7 genotype. iPSCs were electroporated with gRNAs and Cas9 protein. After a week, single clones were assessed for HLA-A and HLA-B expression using flow cytometry. Two gRNAs were employed simultaneously (sgRNA 1.2) and individually (sgRNA1 & sgRNA 2). Clone 5 from cells treated with sgRNA1.2 exhibited heterozygous knockout of HLA A2 expression and partial reduction in HLA BW4 expression. Consequently, clone 5 was expanded and several clones were derived. Flow cytometry analysis revealed homozygous knockout of multiple HLA alleles in all clones except one. To confirm the findings, next-generation sequencing was conducted. Both HLA-A02:01 and HLA-A03:01 were successfully knocked out in two samples: Sg1.2 clone 5 (3) and Sg1.2 clone 5 (5), which will be used as negative controls for T cell activation in further assays. Also, genomic sequence analysis revealed that, as expected, HLA-Cw03:04 and HLA-Cw07:04 remained unaffected by CRISPR editing. Furthermore, HLA-B44 displayed a partial knockout, while HLA-B51:01 remained unedited. This was reflected by a reduction in staining for the HLA-Bw4 epitope at the cell surface. These findings underscore the feasibility and efficacy of CRISPR-based genome editing in tailoring HLA expression profiles in iPSCs, thereby advancing the prospects of regenerative medicine.

7. The role of vcam1 in the niche during development of hematopoietic stem cells.

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During mammalian development, the interaction between hematopoietic stem and progenitor cells (HSPCs) and the fetal liver niche is essential for the programming and expansion of the stem cell pool. In the fetal liver are stromal cells, endothelial cells, hepatocytes, and macrophages. However, the identity of niche cells that provide the necessary signals for correct HSPC development is still debated. The zebrafish equivalent of the fetal liver is the caudal hematopoietic tissue (CHT). The role of integrin alpha 4/beta 1 on HPSCs and its interaction with

the vascular cell adhesion molecule vcam1 in the fetal liver niche has been well established. Our lab and others have shown these adhesion proteins are well conserved in the zebrafish CHT. However, the cell types expressing vcam1 (1a and 1b in zebrafish) and their contribution to HSPC development are not fully understood. We are using multiple approaches to define the expression and role of vcam1 in the CHT niche. We have a new rabbit polyclonal anti-vcam1 antibody that was validated by western blot and loss of reactivity in double vcam1b/1a mutant/morpholino knock-down embryos. We found the anti-vcam1 antibody works well in flow cytometry but not whole-mount immunofluorescence. Therefore, we used whole-mount fluorescent in situ hybridization (RNAscope) in different transgenic backgrounds to determine the specific CHT cell type expressing vcam1 at ~52 hours post fertilization (hpf) during HSPC colonization. We found that endothelial cells and macrophages in the CHT do not express vcam1, contrary to previously published studies that proposed vcam1+ macrophages are required to guide HSPCs into the niche. Instead, we determined that vcam1 is likely expressed by CHT stromal cells. **These findings are significant because they suggest HSPC-stromal cell interactions are critical for HPSC development and programming in the CHT niche.**

8. ENGINEERING THE MULTICELLULAR NICHE FOR T CELL DIFFERENTIATION

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Induced pluripotent stem cell (iPSC) derived T cells show great potential for off-the-shelf immunotherapy1. Methods for differentiating human T cells from iPSCs rely primarily on cocultures, such as the OP9-DLL4 platform2. Feeder-free T cell differentiation platforms have generated clinically relevant iPSC-T cells, yet focus on engineering synthetic niches without understanding co-culture systems3. To determine how niche cells drive T cell development, we modeled cell-cell communication in the human thymus using NicheNet4.

We have predicted ligands that impact the differences between in vitro and in vivo derived T cells 5,6 and investigated their effect in the OP9-DLL4 T cell differentiation platform. We hypothesized that knocking out or activating Il15, Tnf, Cd80, Cxcl12, Il6, Crlf2, Tslp, Btla, Cd40, Cd40lg, Adam17, H2-d1 Or Il7 in the OP9-DLL4s may improve T cell maturation to CD8. We performed an arrayed CRISPR screen to individually knock out each target in the OP9-DLL4 cells. Day 21 T cell progenitors, generated from primary cord blood CD34+ cells, were seeded onto confluent OP9-DLL4 with each target knocked out. CD4 and CD8 expression was assayed via flow cytometry one week later. As expected, knocking out H2-d1 decreased the percentage of CD8 over CD4 T cells. Knocking out known targets (Il15 and Tnf) and unexpected targets (Inhbb, Ocln)

increased the percentage of CD8 over CD4 T cells, while knocking out Tslp resulted in a higher ratio of CD4 to CD8 T cells. Currently, we are exploring the effects of combination knockouts of each of these targets on T cell development.

These data could uncover previously unknown roles for ligands in T cell differentiation and identify targets for manipulation of the differentiation niche. We present a systems-biology approach to understanding the OP9-DLL4 coculture through modeling cell-cell communication and performing a CRISPR screen of computational hits. By learning from current T cell differentiation methods, we can engineer a more biomimetic synthetic thymic niche for manufacturing of more potent iPSC-T cells.

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9. Trimodal single-cell profiling of T-cell acute lymphoblastic leukemia identifies leukemic stem cells.

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T-cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy resulting from the aberrant expression of oncogenic transcription factors (TFs), which triggers the malignant transformation of T-cell progenitors. Despite advances in treatment, T-ALLremains challenging to cure due to the potential for relapse, often attributed to a small subset of cells with stem cell-like properties known as leukemic stem cells (LSCs). These LSCs play a critical role in the persistence and recurrence of T-ALL, primarily because they can evade therapies and spur the disease's regeneration. Their adaptability is central to leukemia's pathology, making these cells particularly elusive targets for both research and therapeutic strategies. Understanding the molecular basis of LSC adaptability is crucial for advancing treatment strategies, yet this knowledge remains elusive. To elucidate these mechanisms, we applied a single-cell trimodal assay capable of profiling RNA, chromatin accessibility, and 164 surface proteins simultaneously in primary tumors from patients with acute leukemia. This advanced technique allowed us to identify and isolate small populations of cells with notable stem cell properties compared to other blasts. Subsequent analysis revealed that these cells had altered their gene regulatory networks to acquire stem cell-like properties. Importantly, these cells were confirmed to function as LSCs, given their ability to engraft, self-renew, and differentiate into leukemic blasts in vivo, thus providing a breakthrough in identifying the elusive LSCs. Our findings not only highlight the cellular complexity of T-ALL but also demonstrate the powerful potential of integrating multidimensional single-cell data to uncover the cellular hierarchies and the key players in the disease process of human T-ALL across its various oncogenic subtypes. Additionally, we have identified potential new targets for therapy that may effectively eliminate LSCs, providing a promising path for the development of more efficient treatments.

10. Loss of integrin α4 Function During Development Leads to Premature Aging of Hematopoietic Stem and Progenitor Cells in Adulthood

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Hematopoietic stem and progenitor cells (HSPCs) reside in a microenvironment that regulates their behavior by interactions with niche support cells. In the mammalian embryo fetal liver HSPCs proliferate and when transplanted have a greater capacity for reconstituting the blood system compared to quiescent adult HSPCs. However, the genetic networks regulating fetal liver HSPCs are poorly understood. To dissect these networks, we are characterizing a viable integrin α4 (itga4) mutant zebrafish model with perturbed interaction between HSPCs and caudal hematopoietic tissue (CHT), equivalent to mammalian fetal liver. To define niche reprogramming mechanisms, we sorted WT and itga4 mutant Runx:mCherry+ HSPCs via FACS after CHT stage at 5 days post fertilization. Bulk RNA-seq detected 286 up- and 49 down-regulated transcripts in itga4 mutant versus WT ($q<0.05$) and GSEA revealed upregulation of inflammatory pathways in itga4 mutant HSPCs. Bulk ATAC-seq discovered 84,236 peaks unique to WT HSPCs and 16,875 peaks unique to itga4 mutant ($q<0.05$) with motif analysis identifying potential regulatory factors of HSPC reprogramming, including ETS and AP-1 factors. To

understand the loss of itga4 function through development and into adulthood, we compared whole kidney marrow (WKM) from young (6-month-old) itga4 mutant and WT adults, and aged (24-month-old) WT adults via split-pool barcoding scRNA-seq. Factors increased in aged WKM, including cebpa, il1b, and nfkbiab, were also increased in young itga4 mutant. Myeloid bias of young itga4 mutant HSPCs was enriched versus young WT (4.46% vs 1.63%, $p=0.0432$). Percentage of mitotic HSPCs was increased in young itga4 mutant versus young WT (47.42% vs 3.83%, $p<0.001$). Together, our data suggests that young adult itga4 mutant HSPCs exhibit features of premature aging after failed CHT niche reprogramming. We will utilize this system and multiomic approaches to dissect developmental networks of HSPC regulation. Our findings could translate into novel approaches for stem cell expansion and therapy.

UW – Madison Stem Cell Graduate Scholar Awardees

11. Microbiome-Driven Type I Interferon Signaling Regulates the Survival of Mature and Progenitor B-cell populations

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Introduction: Our lab has shown that the microbiome depletion on long-term antibiotics impairs steady-state hematopoiesis. We found that both mature and progenitor B-cell populations were strongly and reproducibly suppressed upon antibiotic treatment in mice. We discovered that the microbiome supports normal blood production by inducing basal level of type I interferon (IFN-I) production. However, the mechanism by which microbiome-derived signals regulates B-cell homeostasis remain elusive. We hypothesized that microbiome-driven basal IFN-I production regulates the B-cell survival by activating PI3K pathway.

Methods: We treated mice with an antibiotic cocktail or flavoring alone in the water for 2 weeks *ad libitum*. To determine if suppression on antibiotics is attributed to impaired proliferation, we performed BrdU staining to quantify B cell proliferation via flow cytometry. Next, we tested the effect of the microbiome depletion on B-cell survival by quantifying apoptotic cells and polycaspase activation via Annexin V staining and FLICA, respectively. Lastly, we quantified the expression of genes involved in PI3K pathway via qPCR.

Results: We found that microbiome-driven IFN-I signaling is not required to regulate the cell cycle dynamics but is required for the survival of B-cell populations in blood, bone marrow, and spleen. Our data from the FLICA assay validated the Annexin V staining results by showing increased polycaspase activation in B-cells from antibiotic-treated mice compared to the controls. Furthermore, expression of genes involved in PI3K pathway, *Mapk26* and *Mapk1* was significantly reduced in Abx-treated mice compared to controls.

Conclusions: Microbiome-driven tonic IFN-I signaling activates PI3K pathway to promote B-cell survival.

12. The Sterile Alpha Motif Protein-1 Transcriptional Co-Repressor Inhibits Hematopoiesis

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Hematopoietic stem and progenitor cell (HSPC) differentiation is marked by several intermediate states of progressively restricted lineage-committed cells (e.g., the megakaryocyte-erythrocyte progenitor (MEP)). Transcriptomics studies have highlighted the intricacy of this process, involving significant cell state heterogeneity during differentiation. Within these datasets, a family of related sterile alpha motif (SAM)-containing proteins are elevated in MEPs. We identified that the protein SAMD1 is expressed highly in hematopoietic stem cells (HSCs) and MEPs and is predicted to control key hematopoietic transcription factors such as GATA2. In

other contexts, SAMD1 acts as a transcriptional co-repressor with LSD1 to control transcription. **I hypothesize that SAMD1 is required for transcription and/or signaling during hematopoiesis.** I tested this hypothesis using shRNA knockdown or CRISPR-Cas9 KO systems in human and mouse hematopoietic progenitors. Following SAMD1 knockdown/knockout, I differentiated mouse lineage-depleted bone marrow or human CD34+ cells along the erythroid lineage. SAMD1 knockout increased the frequency of late erythroid progenitors (CD71-CD235a+) by 8-fold. Consistent with the observation that SAMD1 knockout increased erythropoiesis, an RNA-seq analysis identified a cohort of SAMD1 repressed genes were involved in heme metabolism (TRIM10 and TRAK2) and ROS pathways (GLRX2 and GLCM). Conversely, SAMD1-activated genes include those involved in hemostasis and platelet activity pathways (F2RL3 and FLNA). Overall, my results suggest a role for SAMD1 in cell fate.
SAMD1 knockout is embryonic lethal, and no conditional knockout mouse currently exists. To clarify the role of SAMD1 in hematopoiesis, we conducted competitive transplant experiments in mice using shRNA knockdown HSCs. Samd1 knockdown versus control HSCs revealed an increase in HSC repopulation with 30% more CD45.2+ after 16 weeks. Currently, we are testing possible SAMD1 transcriptional mechanisms in relation to LSD1 using ChIP-seq and co-immunoprecipitation assays. Both SAMD1 and LSD1 are commonly upregulated in acute myeloid leukemia (AML), and high expression is correlated with poor prognosis. Linking Samd1 function to signaling and transcriptional mechanisms opens the door to translational avenues for studying the contribution of Samd1 in hematologic pathologies.

13. Runx factors regulate ex vivo haematopoietic stem cell expansion

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Multipotent self-renewing haematopoietic stem cells (HSCs) are important for human health because they can reconstitute the entire blood and immune system. HSCs are also thought to be essential for haematopoietic homeostasis and haematopoietic system dysfunction can be fatal. HSCs are extremely rare with just ~1:30,000 in the adult bone marrow. They are also difficult to grow *ex vivo*, with serum albumin-based cultures only supporting short-term maintenance. Because of these limitations, it has been difficult to comprehensively study the mechanisms that balance HSC self-renewal and differentiation. New polyvinyl-alcohol-based cultures permit long-term expansion of mouse HSCs *ex vivo*. This has made molecular and genetic analyses much more permissible. Here, we have used a genome-wide CRISPR knockout screen to identify novel genetic regulators of *ex vivo* HSC expansion. From this screen, we identified Runx transcription factors as negative regulators of *ex vivo* HSC expansion. We have validated these findings using CRISPR-based gene knockouts, *ex vivo* expansion assays, and *in vivo* transplantation assays and made the novel discovery that Runx factor ablation promotes HSC fitness. Taken together, our data suggest that Runx factors act to limit to *ex vivo* HSC expansion and identifies a potential axis to further improve *ex vivo* HSC expansion approaches.

14. Investigating the role of *SF3B1* mutation-mediated mis-splicing of *UBA1* in MDS

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Myelodysplastic syndromes with ring sideroblasts (MDS-RS) are a low-risk MDS subtype that originate in hematopoietic stem cells and are predominantly defined by mutations in the *SF3B1* splice factor and a clinical profile of refractory anemia. Treatment of MDS-RS is largely limited to mitigating the anemia through transfusion or use of erythropoiesis-stimulating agents, and allogeneic stem cell transplants remain the only curative option. An incomplete understanding of the mis-splicing landscape and its molecular consequences in shaping the disease phenotype, as well as the lack of accurate model systems, has hampered the development of new treatments. To identify novel RNA mis-splicing events, we employed full-length RNA sequencing of bone marrow CD34+ cells from MDS-RS patients, followed by functional characterization using a patient-derived induced pluripotent stem cell (iPSC) model. Cohort analysis among splice factor-mutant patients identified significant, *SF3B1* mutation-exclusive mis-splicing of the gene ubiquitin-like modifier activating enzyme 1 (*UBA1*), which encodes the master enzyme of the cellular ubiquitination cascade. To evaluate the effect of *UBA1* mis-splicing on protein function, K562 cells were transfected with cDNA constructs encoding mis-spliced or wild-type *UBA1*. While both led to mRNA production, the mis-spliced *UBA1* construct failed to generate protein. Further, iPSC lines generated from mutated and wild-type cells from MDS-RS patients were differentiated into hematopoietic cells *in vitro*. RNA sequencing identified the same mis-splicing event of *UBA1* in the *SF3B1*-mutated line and its absence in *SF3B1* wild-type cells. To determine whether lower levels of functional UBA1 protein sensitize cells to UBA1 inhibition, hematopoietic cells from both iPSC lines were treated with the selective UBA1 inhibitor TAK-243. Interestingly, cell death was induced in *SF3B1*-mutated cells at significantly lower doses compared to wild-type *SF3B1*. In conclusion, we have identified the novel and significant mis-splicing of *UBA1* in *SF3B1*-mutated patients which increases mutant cell sensitivity to *UBA1* inhibition, providing a new avenue to improve treatment of MDS-RS.

GENERAL POSTER SESSION

15. Identifying synthetic hydrogels for iPSC-derived cardiomyocyte maturation

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Induced pluripotent stem cells (iPSCs) have successfully been differentiated into cardiomyocytes (iPSC-CMs), creating opportunities in disease modeling, drug testing, or cardiac regeneration. iPSC-CMs are traditionally differentiated on an exogenous extracellular matrix, namely Matrigel. This results in high variability in differentiation, xenogeneic contamination, and limited cell maturation. We show that fully-defined synthetic materials can be engineered to better support reproducible maturation of iPSC-CMs.

By varying the adhesion ligand type and concentration (fibronectin- and laminin-derived) and the hydrogel shear modulus, we developed an optimized polyethylene glycol (PEG) based hydrogel formulation. Our optimization targeted PEG hydrogel formulations that maximized iPSC-CM maturation. For this, we assessed gene expression of sarcomere proteins using rt-qPCR, measured Ca2+ handling efficiencies via fluorescence imaging, and assessed the cell's optical redox ratio via optical redox imaging. To rapidly identify our optimized hydrogel formulations, we defined a maturation score which describes the overall iPSC-CM maturation by combining each measurement. To effectively maximize this score, we used an optimization model to identify regions of interest in our parameter space for further screening.

Our screening approach identified one hit PEG hydrogel formulation, which outperformed iPSC-CM maturation on Matrigel, during our first screen. Using our optimization model, we identified hydrogel formulations which outperformed those tested in the first screen. From these, we also identified one additional hit PEG hydrogel formulation. These two PEG hydrogel hits could be used as an alternative for iPSC-CM differentiation and maturation. Our optimization model could also be used to identify microenvironmental conditions for other cell types or desired cell behavior.

16. Identifying novel cardiac regeneration enhancers by utilizing computational analyses and transgenic assays

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Heart regeneration relies on the reconstruction of gene regulatory networks (GRNs) in response to cardiac injury, which is orchestrated by tissue regeneration enhancer elements (TREEs). Identifying groups of TREEs exhibiting similar features will provide a base for elucidating GRNs that control heart regeneration. We previously dissected a cardiac regeneration enhancer in zebrafish to determine the regulatory mechanisms governing heart regeneration. The cardiac leptin b regeneration enhancer (cLEN) exhibits injury-inducible activity near the wound in the heart, which is conferred by multiple injury-activated regulatory elements distributed

throughout the enhancer. Our analysis also found that cardiac regeneration enhancers are actively repressed in the absence of injury, demonstrating dual regulatory mechanisms of cardiac TREEs. Our extensive transgenic assays identified a short 22-bp DNA sequence containing a key repressive element responsible for maintaining the inactivation of cLEN in uninjured hearts. To uncover a group of TREEs similar to cLEN present in the genomes of zebrafish, mice, and humans, we devised a strategy to identify cLEN-like enhancer candidates by analyzing sequence similarity, evolutionary conservation, and epigenomic and transcriptomic profiles. For selected hits, we performed transgenic assays in zebrafish to determine which candidates are functional TREEs. Our transgenic assays identified multiple enhancers in the zebrafish and mammalian genomes that exhibit injury-inducible activation in hearts. Identifying additional regeneration enhancers across species will expand our understanding of the regulatory mechanisms underlying heart regeneration and lead to the identification of potential targets for improving heart repair.

17. Mechanisms regulating induction of blood-brain barrier properties in a human pluripotent stem-cell derived model system

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Induction of blood-brain barrier (BBB) properties in central nervous system (CNS) endothelial cells during human development is incompletely understood and our knowledge of this process is derived mainly from animal models. In vitro BBB models derived from human pluripotent stem cells (hPSCs) can be used to study human BBB development and cerebrovascular disease. Human-derived in vitro models can be used to probe the relative importance of specific signaling pathways, including Wnt/β-catenin and Notch, on induction of BBB properties in naïve endothelium. We differentiated endothelial progenitor cells (EPCs) expressing both CD31 and CD34 from hPSCs and treated the hPSC-EPCs with CHIR99021 to activate the Wnt/β-catenin signaling cascade and overexpressed the Notch1 intracellular domain (N1ICD) to simulate signaling through the Notch1 receptor. We find that co-activation of Wnt/β-catenin and Notch1 signaling resulted in simultaneous upregulation of GLUT-1, a BBB-enriched glucose transporter, and downregulation of two vesicular transcytosis-related structural proteins, PLVAP and caveolin-1. These findings suggest that the combination of these two signaling inputs yields induction of important barrier properties, including expression of a nutrient transporter and reduction of transcytosis-associated structural protein expression. Transcriptomic analyses recapitulate the changes in nutrient transporter and transcytosis-related gene expression, including upregulation of Gene X, which is implicated in negative regulation of transcytosis at the BBB. RNA-seq also confirms the maintenance of endothelial identity of the N1ICD-overexpressing cells. Identification of pathways with potential importance for developmental specification of BBB properties in humans will advance efforts to model the human BBB in vitro for the study of CNS drug delivery and neurovascular disease.

18. Single-cell RNA sequencing reveals distinct maturation and motility signatures in GATA1 and PTP1B knockout iPSC-derived neutrophils

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During development, neutrophils undergo sequential maturation stages, from precursors to mature neutrophils before egressing to the circulation and tissues. Induced pluripotent stem cell (iPSC)-derived neutrophils (iNeuts) offer a new tool to understand neutrophil development. Previous studies have shown that iNeuts with GATA1 deletion (GATA1-KO) generate more mature neutrophils, while PTP1B deletion (PTP1B-KO) results in more immature neutrophils than WT. There remains a lack of understanding of the heterogeneity and transcriptional changes between different developmental stages within iNeuts and its derivatives. This study aims to characterize heterogeneity, focusing on the maturation and motility features of GATA1-KO and PTP1B-KO iNeuts compared to WT using single-cell RNA sequencing (scRNA-seq). By mapping from publicly available primary human neutrophil datasets using computational label transfer methods, we show the extent of maturation transcriptional signature distinction between three iNeuts lines, explaining previous morphological and flow cytometry observations. We will present the gene regulatory network underlying the shift from immature states in WT to almost completely mature neutrophils in GATA1-KO. We will also show gene set enrichment analysis that is associated with the decrease of motility in GATA1-KO, and the increase of motility and antimicrobial function in the PTP1B-KO. We anticipate that this work will help identify the transcription factors regulating iNeuts development and provide ideas to generate neutrophils in vitro toward a specific state. Given the emerging evidence of novel functions of neutrophil subsets in humans, our work will provide a valuable framework for using iNeuts in studies of infectious diseases or cancer.

19. Making Cells Make Themselves: Directed Differentiation of hPSCs to Atrial Cardiomyocytes Using Single Cell-Level Reprogramming

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Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) are currently used in disease modeling, drug discovery, and treatment of cardiovascular diseases (CVDs), the leading cause of death globally. However, the potential of hPSC-derived CMs in biomanufacturing is limited by batch-to-batch variability, especially for applications that require large numbers of CMs. hPSC-derived CMs are created by modulating the WNT pathway. Protocols for chamber specific atrial CMs (aCMs) also use retinoic acid to induce atrial fate. aCM protocols are also prone to low yields. This limits the potential of hPSC-derived aCMs in modeling disease including the most common fibrillation found in clinic, atrial fibrillation (aFib).

Current protocols generally provide external signals to guide hPSCs through developmental stages. However, hPSC-derived CMs are prone to heterogeneity within the differentiation culture due to differences in developmental state and cell cycle. This heterogeneity results in a mismatch between cell state and differentiation cues resulting in a mixed population of cell types. This limits the potential use of hPSC-derived CMs in large scale biomanufacturing. We hypothesize that using gene circuits to sense the primed state of differentiating cells and provide signaling at a single-cell level will reduce heterogeneity created by a mismatch between individual cells' differential state and external signals. We propose using integrated genetic circuits to provide signals at a single cell level at the optimal developmental state to induce fate changes during CM differentiation to reduce heterogeneity. We also hypothesize this approach will improve reproducibility of hPSC differentiation though the reduction in heterogeneity.

Improving heterogeneity and reproducibility will increase practicality of CM and aCM differentiations for wide scale use of hPSC-derived CMs for modeling CVDs and aFib.

20. Histone demethylase control of primordial germ cell specification

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During embryonic development approximately 40 cells are designated as Primordial Germ Cells (PGCs) and are destined to become sperm or egg. These cells undergo massive epigenetic remodeling, which is not seen in the surrounding somatic cells. The repressive histone modification H3K9me2 is depleted during PGC specification. In parallel, DNA is demethylated in two waves: a global erasure followed by a loci specific depletion. The only locations to escape this first wave of global erasure are a few germ cell specifying genes, parental imprinting loci, and retroviral elements. How these loci are protected during this process is still unknown. The enzymes that catalyze this epigenetic remodeling are H3K9me2 specific histone demethylases, KDM3A and KDM3B, and the DNA demethylases, TET enzymes. Our prior work from somatic cell reprogramming to induced pluripotent stem cells, indicated that there is coordinated removal of H3K9me2 and DNA methylation. We hypothesize that a similar mechanism is at play during germ cell development. Therefore, we have established an in vitro differentiation model of embryonic stem cells to primordial germ cell like cells (PGCLCs) in which we can rapidly eliminate the KDM3 proteins using a “degron” tag. Using this tool, we have found that complete removal of the KDM3 proteins compromises PGCLC development. The results from this work will inform germ cell specification and fertility.

21. The Role of GABA Receptor Gabbr1 during Hematopoietic Aging

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Hematopoietic stem and progenitor cells (HSPCs) can self-renew and replenish the entire hematopoietic system and are therefore clinically valuable. During aging, HSPCs are marked by reduced regenerative capacities and lineage-biased differentiation. The decrease in lymphocyte production by HSPCs leads to a decline in immune function. However, the mechanisms underlying HSPC lineage biases during aging are largely unknown. Previously, our lab generated a gamma aminobutyric acid (GABA) type B receptor subunit 1 (Gabbr1) knockout mouse model that had reduced B lymphocyte production and stem cell fitness after transplantation. We found that GABA-GABBR1 signaling has a role in HSPC differentiation into B cells. Addition of GABBR1 agonist to HSPC/OP9 stromal cell co-culture increased B cell production by 3.57-fold ($p = 0.033$). We also identified B cells and bone marrow endothelial cells (BMECs) as key sources of GABA based on their expression of glutamic acid decarboxylase (GAD) enzymes that produce GABA. We blocked GABA production from B cells and BMECs by conditionally knockout Gad in these two cell types. Blockade of B cell and BMEC-derived GABA lead to a 14% reduction in B lineage cell in the peripheral blood ($p = 0.021$). Interestingly, we detected a 1.47-fold elevation in GABA level in aged male mice despite the reduction in B cell and BMEC number. Furthermore, we found aged HSPCs lost responsiveness to GABBR1 agonist stimulation in OP9 co-cultures. This may be explained by the smaller fraction of HSPCs we found express Gabbr1 in aged bone

marrow with a 2.58-fold decrease compared to adults (p = 0.06), or because of other unknown intrinsic differences in aged HSPCs. Our results suggest dysregulation of GABA-GABBR1 signaling in aged bone marrow that may contribute to the age-related decline in B cell production. Future investigation will explore the molecular mechanism of the GABA-GABBR1 signaling pathway, and identify potential targets to improve hematopoietic function during aging.

22. Improving Non-Human Primate Hematopoietic Differentiations Using De Novo Non-Human Primate scRNAseq Datasets

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Engraftable hematopoietic stem cells (HSCs) are the elusive holy grail that labs studying In vitro hematopoiesis are looking to generate. Single cell RNA sequencing (scRNAseq) datasets on the earliest fetal HSC populations have been an indispensable reference resource for researchers attempting to improve the transcriptomic phenotype of their in vitro differentiations. However, for our purpose of studying non-human primate (NHP) hematopoiesis, these datasets do not exist. To fill this gap, we set out to create the first NHP fetal liver mononuclear cell (FL-MNC) scRNAseq dataset, so we could accurately compare and improve our NHP iPSC to hematopoietic progenitor differentiations. We isolated four samples of FL-MNCs from two species of NHPs (Macaca fascicularis and Macaca mulatta) along with hematopoietic progenitor cells generated in vitro using our current differentiation and an improved method. We generated the single cell RNA sequencing libraries in house using PIPseq technology that were then sent off for sequencing. By comparing the NHP scRNAseq data to human FL-MNC scRNAseq datasets we identified all expected FL-MNC populations. We were able to show that the HSC specific gene phenotype identified in humans is conserved in NHPs. Additionally, by comparing the In vivo HSCs to In vitro, we were able to show that our improved NHP differentiation more accurately recapitulates the fetal liver HSC phenotype compared to our current differentiation system, we also identified a previously unknown cell population within our in vitro differentiations recapitulating the intra-aortic hematopoietic cluster phenotype seen in vivo during early hematopoiesis. Finally, we identified genes whose expression is currently lacking in our in vitro differentiations in comparison to in vivo HSCs. Going forward, we plan to use these genes to inform future adaptations to our NHP differentiation.

23. Advancing NHP Model for Evaluation of Pluripotent Stem Cell Technologies for HIV Immunotherapies

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Adoptive T cell therapies with in vitro expanded genetically modified T cells have been considered a valuable strategy to treat and cure HIV. A renewable source of human T cells from induced pluripotent stem cells (iPSCs) would further facilitate and broaden the applicability of

these therapies. To enable evaluation of iPSC-based technologies in a preclinical HIV infection model, we explored the feasibility of production of CCR5-edited and anti-HIV CAR T and NK cells from Mauritian cynomolgus monkey iPSCs generated from fibroblasts and peripheral blood T cells. Using CRISPR-Cas9 we successfully introduced deletion within exon 2 of CCR5 including a 24-bp deletion region that was previously found to prevent functional CCR5 expression in NHPs. We demonstrated that T cells and macrophages produced from CCR5-edited iPSCs did not support the replication of the CCR5-T cell-tropic SIVmac239 and macrophage-tropic SIVmac316 simian immunodeficiency viruses, respectively. However, we noted an impaired capacity of iPSCs generated from T cells (T-iPSCs) to re-differentiate into T cells, especially following biallelic CCR5 disruption. In addition, we established MCM iPSC lines with anti-HIV CD4-MBL CAR inserted into AAVS1 locus under CAG promoter or CCR5 locus under MND promoter. CD4-MBL CAR iPSCs were used succefully to generated T and NK cells which retain CAR expression following differentiation. MCM iPSC-derived CD4-MBL CAR NK cells demonstrated superior cytotoxic activities against HIV1 envelope expressing CHO cells. Overall, these studies provide a platform for further exploration of AIDS therapies based on gene-edited iPSCs in a NHP model.

24. Identifying the gene regulatory networks that control the cell fate decisions during reprogramming to pluripotency.

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Reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) holds transformative potential for regenerative medicine as they can give rise to any cell type in the body and can be therapeutically targeted with genome editing tools. However, the reprogramming process is inefficient as few starting cell populations can successfully complete the acquisition of pluripotency. Defining the gene regulatory network (GRN) dynamics during the reprogramming process could provide insight into improving reprogramming efficiency, but is a formidable challenge. Here we employ high-throughput computational and experimental methods to predict GRNs and key regulators of the reprogramming process.

We first applied Single Cell Multitask Network Inference algorithm (scMTNI), a GRN inference algorithm, to a previously collected single cell multi-omic time course during reprogramming. scMTNI integrates cell lineage structure, scRNA-seq, and scATAC-seq measurements to enable joint inference of cell type-specific GRNs. scMTNI predicted GRNs specific to successfully reprogrammed and non-reprogrammed cell populations. Notably, by examining how the networks change between those two populations, we identified ~80 regulators that could be important in directing cell fate decisions.

To validate our findings, we adapted the Perturb-seq approach that combines CRISPR screening with scRNA-seq profiling to enable multi-modal measurements. We constructed a new gRNA delivery vector that allows direct capture of delivered guide RNAs by both scRNA-seq and scATAC-seq methods through specific sequence features. Our pilot experiment showed that we could successfully deliver and capture gRNAs without losing diversity and can achieve nearly an 80% reduction in targeted gene expression levels using our new vector. We plan to apply our new approach to a reprogramming time course experiment while perturbing each of the predicted 80 regulators. We expect to uncover key regulators direct that cells to successful and

unsuccessful fates. Manipulation of Identified key regulators can help to achieve desired efficiency of reprogramming.

25. Reseeding during early phases of small molecule-based human pluripotent stem cell cardiomyocyte differentiation increases cardiomyocyte purity and enables cryopreservation

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have the ability to transform the treatment of heart disease, which is the leading global cause of death. Seminal preclinical studies have demonstrated that hPSC-CMs can remuscularize the heart in animal models of myocardial infarction (heart attack) and heart failure leading to several clinical trials worldwide. Moreover, hPSC-CM models are actively being used to study drug cardiotoxicity, human development, and human disease. Despite their promise, hPSC-CM differentiation protocols are plagued by batch-to-batch and line-to-line variability in differentiation outcome for CM purity (cardiac troponin T+ cells) by flow cytometry. Using expected optimal values for seeding density and CHIR99021 concentration, we show that average CM purity for small molecule-based CM differentiation is only ~50% CMs. In our laboratory, only ~15% of CM differentiations achieve a high purity threshold of 70% CMs, which has been established to improve contractility in transplantation studies and engineered cardiac tissues in vitro. In this work, we describe a method to improve CM purity by lifting and reseeding early progenitors between the mesoderm and cardiac progenitor stage of CM differentiation. Using this method, CM purity is increased by 10-15% across multiple cell lines without affecting contractile function (automated video assessment) or structure (sarcomere length). Moreover, we demonstrate that mesoderm and cardiac progenitor stage cells specifically are amenable to cryopreservation with similar improvements in CM purity after resuming differentiation. Lastly, this method also enables the precise introduction of defined extracellular matrix components during developmentally relevant time windows. In summary, we present a method to increase CM purity for small molecule-based hPSC-CM differentiation and demonstrate that specific early progenitors are amenable to cryopreservation.

26. CIN Induces Interphase Micronuclei Formation in HoxB8 Immortalized Myeloid Progenitor Stem Cells.

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Hematopoietic stem and progenitor cells (HSPCs) rapidly proliferate to drive bone marrow expansion during embryonic development and during stress hematopoiesis in adults. In addition, rapid HSPC proliferation enables complete bone marrow reconstitution in the bone marrow transplant setting. During the resulting mitoses, HSPCs must segregate chromosomes to daughter cells with complete fidelity. Errors lead to the random loss or gain of whole chromosomes or portions of chromosome arms, leading to acquisition of cytogenetic abnormalities and micronuclei formation, in a process called chromosomal instability (CIN). We hypothesize that transient induction of CIN in HSPCs leads to cytogenetic abnormalities and micronuclei formation, inducing bone marrow failure and predisposition to bone marrow

malignancies through chronic activation of the innate immune system. Here, we use the small molecule Mps1 inhibitor reversine to transiently induce CIN in HoxB8-immortalized murine myeloid progenitor cells. Reversine treatment leads to a dose-dependent increase in missegregated metaphase chromosomes, anaphase lagging chromosomes, and interphase micronuclei compared to DMSO control. CIN occur even at reversine concentrations that do not adversely impact cell viability. We show that a subset of resulting micronuclei are positive for cGAS, indicating activation of the cGAS-STING innate immune response pathway. Ongoing studies will examine the downstream consequences of micronuclei-induced cGAS-STING activation on HSPC function and test whether the hematopoietic transcription factor GATA2 works to suppress CIN or micronuclei-induced innate immune signaling.

27. Increasing the yield of CNS-like endothelial cells from hPSCs with timed VEGF media supplementation

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When developing human in vitro blood-brain barrier (BBB) models, hPSCs offer a scalable alternative compared to primary cells. When differentiating hPSCs to endothelial cells (ECs) for BBB modeling, it is important to consider the acquisition of CNS-specific EC attributes, including increased transendothelial electrical resistance, decreased permeability, increased tight junction protein expression, and expression of certain transporters. One published method to differentiate hPSCs into CNS-like ECs involves first differentiating endothelial progenitor cells (EPCs) that can be further induced by Wnt signaling to produce CNS-like ECs. However, the process to differentiate EPCs can be somewhat inefficient, and we wished to optimize EPC yield and purity. To differentiate hPSCs into EPCs, undifferentiated hPSCs are expanded on Matrigel-coated plates for three days then transferred into LaSR medium containing 6-7 uM CHIR99201 for 48h. Subsequently, medium is switched to basal LaSR medium for an additional 72h of outgrowth before CD31+EPC purification by MACS. Often this protocol results in <15% CD31+EPCs pre-MACS from a heterogenous cell population and post-MACS purity of 30-80%. In an effort to increase both purity and yield of EPCs, the differentiation protocol was altered to supplement basal LaSR medium with VEGF during the outgrowth phase and prior to MACS recovery of CD31+EPCs. Media supplemented with VEGF resulted in a significant increase in percentage of CD31+EPCs present in the total cell population pre-MACS (up to 40%) when tested in 3 hPSC lines (IMR90-C4, H9, 19-9-11). Post-MACS, cell populations had consistent, elevated EPC purity (up to 95% CD31+EPCs). Additionally, EPCs generated in VEGF-containing medium cultured in the presence of CHIR99021, exhibited expected CNS-like EC characteristics including an increase in expression of GLUT-1 and Claudin 5, and decreased permeability. Thus, media supplementation with VEGF increases yield and purity of CD31+ EPCs following differentiation, facilitating production of CNS-like ECs for BBB modeling applications.

28. Defining DNA methylation-regulated molecular mechanisms that control cranial neural crest cell biology in orofacial morphogenesis, and cleft pathogenesis

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Among the most common human birth defects, orofacial clefts (OFCs) are now widely recognized to result from complex gene-environment interactions. Cranial neural crest cells (cNCC) are a transient, highly proliferative multipotent stem cell population that form the connective tissue of the head and face. We recently demonstrated that DNA methylation is required for the proliferation and subsequent differentiation trajectory of cNCCs, and that its disruption during early orofacial morphogenesis causes OFCs in the mouse. The studies described here were directed at defining the molecular mechanisms by which DNA methylation regulates cNCC biology during orofacial morphogenesis and OFC pathogenesis. Maxillary mesenchyme tissue from Dnmt1 conditional knockout and control embryos was isolated by microdissection at progressive embryonic stages during initial cleft pathogenesis. Then, whole-genome DNA methylation and RNA-sequencing were conducted to identify correlated methylome-transcriptome changes. Principal component analysis (PCA) from both DNA methylation and RNA-seq data demonstrated that samples cluster primarily by genotype. Analysis of RNA-seq data to-date identified several biological pathways dysregulated following Dnmt1 deletion in the cNCC. Sterol biosynthesis was among the intriguing GO terms identified as it was recently implicated in the regulation of cNCC differentiation, suggesting this as a putative DNA methylation regulated upstream mechanism. Ongoing examination of additional time points and complementary mouse OFC models will further define DNA methylation regulated mechanisms that drive cNCC biology and contribute to OFC pathogenesis.

29. Establishing GATA2-Dependent Hematopoietic Signaling Networks to Decode Pathogenic Mechanisms

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Human GATA2 genetic variation causes GATA2 deficiency syndrome, which frequently leads to bone marrow failure and acute myeloid leukemia. Since there are many questions regarding how GATA2 controls genome function and hematopoietic signaling, ascribing the functional significance of many variants can be challenging. We conducted multiomics (CUT&Tag, ATAC-seq, RNA-seq) with genetic rescue in murine Gata2 -77-enhancer-deleted (GATA2-deficient) myeloid progenitors (hi-77-/-) that have ~75% lower GATA2. We compared how GATA2 and a human germline GATA2 disease variant (9 amino acid insertion between the two zinc fingers) function genome-wide. GATA2-deficiency in hi-77-/- cells upregulated Interferon-gamma and Toll-like receptor signaling components and altered expression of cytokine receptor genes, including Il6st and Il6ra, which encode cytokine receptor subunits IL6ST and IL6RA. Elevated IL-6/STAT3 signaling was reversed by rescue with GATA2 but not the variant. ATAC-seq revealed accessible chromatin in hi-77-/-, but not wild type, progenitors, at -24 kb and -30 kb upstream of Il6st. Surprisingly, despite the 75% decrease in GATA2, CUT&Tag revealed GATA2 occupancy at the -24 kb site in hi-77-/-, but not wild type, progenitors. PU.1 also occupied the -24 kb site only in hi-77-/- cells. Since links between GATA2-deficiency and cytokine signaling had not been established, we asked whether these distal Il6st sites were enhancers that enable IL-6 signaling.

Using CRISPR-Cas9-mediated gene editing, we deleted these sequences in hi-77-/- cells. Compared to the hi-77-/- control, deleting the enhancers decreased Il6st expression 13-fold (P < 0.0001) and lowered IL-6/STAT3 signaling by 85% (P < 0.0001). These results support a model in which GATA2 deficiency engages PU.1, which functions through the enhancers to elevate Il6st expression and IL-6 signaling. As IL6ST is shared by other IL-6 family receptors, including IL-11 and IL-27, studies are ongoing to test the hypothesis that elevated levels of the shared subunit may create complex signaling network alterations.

30. Quantitative mass spectrometry approaches revealed new role of MLL Partial Tandem Duplication (PTD) in Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is characterized by abnormal and rapid proliferation of hematopoietic cells of the myeloid lineage. The histone-3-lysine-4-methyltransferase gene, Mixed Lineage Leukemia 1 (MLL) plays a crucial role in cell fate decision during hematopoiesis and is frequently mutated in patients with AML and myelodysplastic syndrome (MDS). While MLL chimeric fusion proteins have been extensively studied in the context of leukemia, oncogenic mutants with Partial Tandem Duplications (PTDs) of the MLL N-terminal DNA binding domain have not been characterized at the molecular level. In particular, the mechanism through which MLL-PTD, a mutant that differs from MLL chimeric fusion oncogenes in that it has a fully preserved methyltransferase domain, contributes to leukemogenesis is unknown.

Using a combination of cell biology, proteomic approaches in leukemic cell lines, and patient-derived xenograft models (PDX) of AML, we discovered that several copies of the PTD duplication can occur in leukemic cells. Also, our results suggest that the total copy number of C-terminus and N-terminus is lower in leukemic cells compared to cells expressing MLL-WT. We also developed and validated a new antibody specific to the most common partial tandem duplication (exon 6-2). At the genomic level, preliminary results showed that MLL-Nterminus PTD bindings overlap with the MLL-Cterminus and also have unique binding at the genome-wide distribution, suggesting the capacity of Nterminus PTD to aberrantly bind to the genome on its own. In addition, we detected a decreased interaction between the MLL-Cterminus and the WRAD complex in MLL-PTD-expressing cells, which usually greatly increases the methyltransferase activity. Thus, our results suggest a novel mechanism whereby low protein expression of MLL-PTD combined with a defect of its methyltransferase function and potential unique binding of MLL-Nterminus PTD on the genome would lead to the deregulation of gene expression and would underlie the leukemogenic process in AML.

31. The Beta Cell "Invisibility Cloak" – Developing Stem Cell Derived Pancreatic Beta Cells that are Protected from the Immune System

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Introduction: Type 1 diabetes is an autoimmune disorder that destroys pancreatic beta cells leading to insulin deficiency and hyperglycemia. While pancreas and islet cell transplantation are effective in controlling glycemia, challenges exist, including the need for life-long immunosuppression to prevent allograft rejection and autoimmune reoccurrence. We have genetically engineered a human embryonic stem cell (hESC) line that is devoid of immunostimulatory HLA Class I and II expression (double knockout, DKO) and expresses doxycycline-inducible T cell inhibitors, CTLA4Ig (iC) and PD-L1 (iP). We hypothesize these DKO;iCP hESCs can be differentiated to phenotypic and functional stem cell-derived islet-like clusters (SCILCs) and that they are capable of insulin secretory function similar to human cadaver islets.

Methods: DKO;iCP hESCs were differentiated to SCILCs based on an established protocol. Differentiated SCILCs were then transplanted under the kidney subcapsule of diabetic immunodeficient mice receiving doxycycline-laced food. Post-transplant, blood glucose was monitored and serum was collected weekly. Serum was used to measure human C-peptide, an indicator of insulin production.

Results: Here we report that DKO;iCP hESCs can be successfully differentiated into SCILCs with similar morphology, and insulin secretory function to human cadaver islets. Mice transplanted with SCILCs demonstrated higher C-peptide levels compared to diabetic control mice over 8 weeks post-transplant. Fasting blood glucose levels of mice transplanted with SCILCs were similar to those transplanted with human cadaver islets. SCILC grafts collected 12 weeks post-transplant expressed CTLA4Ig and PD-L1 within the kidney.

Discussion: Our results demonstrate the ability to differentiate genetically modified hESCs to functional SCILCs that are able to control hyperglycemia following transplantation. Our studies will continue to examine the ability of these grafts to evade the immune system using a humanized mouse model. These genetically modified SCILCs may provide a scalable cell source for therapeutics that can reverse diabetes while preventing immune rejection.

32. Defining cell type-specific gene regulatory network dynamics of human hindbrain differentiation from single cell RNA-sequencing time course

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During human nervous system development, a precise temporal coordination of gene regulatory processes is needed for differentiation and commitment of diverse cell types of the hindbrain and spinal cord. These structures arise from neuromesodermal progenitor cells (NMP), which differentiate to form neural stem cells (NSC), before diverging to either dorsal or ventral progenitors. However the key transcription factors and gene regulatory networks that drive the transitions between these cell types are not completely known. To overcome these deficits, we used a modular differentiation methodology to generate cells at the NMP, NSC, dorsal progenitor, and ventral progenitor stages from human pluripotent stem cells and measured the

transcriptomes at the single cell level from these four stages. We defined nine cell subpopulations from these data and connected these subpopulations to establish a differentiation lineage tree. We identified gene expression programs capturing coordinated expression state changes along the lineage trajectory as cell type specification progressed. Finally, we defined gene regulatory networks for each subpopulation in the lineage tree. We compared the regulatory networks across the nine cell subpopulations and found subtle but meaningful variation between the subpopulations. These network comparisons identified genes that gained or lost regulatory connections along the lineage tree, and of those connections we identified the top transcription factors for each of the subtypes. Our approach recovered known marker genes for the NMP, NSC, dorsal progenitor, and ventral progenitor cell-types as well as several novel regulators that can be followed up with functional validation experiments to increase our understanding of early human hindbrain development. These findings can help uncover mechanisms underlying early human hindbrain development and provide insights into how this process can be disrupted in early developmental disorders that can be leveraged to improve directed differentiation protocols for basic science and translational needs.

33. A thymocyte-humanized mouse model for assessing the Down Syndrome immune response

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Down syndrome (DS) is a condition in which an extra copy of chromosome 21 is usually obtained by a meiotic error in the gamete, occurring in approximately 1 out of every 100 to 1,000 annual births. Those born with this condition are predisposed to a wide range of health ailments, including, but not limited to, intellectual disability, congenital heart disease, Alzheimer's, leukemia, and immune system deficiencies. Current DS literature is dominated by cognitive studies with a significant focus on neuron function. However, it is also imperative to address the unique immunobiology of DS patients as they have an increased susceptibility to infections, cancer, and autoimmune diseases. Some studies point to DS exhibiting signs associated with

premature aging of the immune system such as increased prevalence of natural killer cells, oxidative stress, and thymus senescence. Furthermore, triplication of chromosome 21 and the associated gene-dosage effects have been implicated in DS phenotypes creating significant variability in DS disease severity. Thus, personalized stem cell therapies would be optimal as stem cell transplantation could potentially regenerate damaged tissues caused by DS immune dysfunction. However, little is known about transplant tolerance in DS patients, and the current mouse model, Ts65Dn, is unable to fully represent the immunophenotypes of DS.

In our study, we have developed a new DS humanized mouse model to study DS pathogenesis and immunobiology, where PSC-derived cardiovascular therapies will also be utilized to determine transplant response and efficacy. Our model allows us to assess transplant allorejection and tolerance to these cells in vivo. Our data show that the DS model is resistant to Graft-versus-host disease (GvHD) and has an increased survival rate compared to conventional euploid peripheral blood-based humanized mice. These findings will allow us to better understand the complex immunobiology of DS patients and potential treatments.

34. ETV2 mRNA Delivery Via Ionizable Lipid Nanoparticles to Direct Induced Pluripotent Stem Cells to a Non-Barrier Endothelial Fate

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Classical pluripotent stem cell differentiation methods rely on the use of small molecules and proteins to direct cell fate, which are costly, lengthy, and inefficient. These methods often involve multiple chemical induction steps and may require inefficient cell sorting techniques. More recently, delivery of transcription factors (TFs) to reprogram cell state has emerged as an alternative means of directing cell fate. Delivery of reprogramming TFs has traditionally utilized randomly integrating virus, which presents the risk of oncogenic mutations, impeding therapeutic applications. With the emergence of COVID-19, ionizable lipid nanoparticle (iLNP) platforms have also been adopted to deliver messenger RNA (mRNA) encoding TFs to direct cellular reprogramming. This type of direct cellular reprogramming would allow researchers to circumvent aforementioned manufacturing challenges. The ETS Variant 2 TF (ETV2) acts as a master regulator of vascular endothelial fate. Expression of this transcription factor has been demonstrated to be sufficient to direct cells directly to an endothelial fate. Delivering this TF to stem cells via iLNPs, endothelial cells can be efficiently generated without using classical methods that rely on small molecules or recombinant. Additionally, by using mRNA delivered by iLNPs, we can induce cell fate change more robustly, efficiently, and uniformly than current fate engineering strategies such as lentiviral transduction.

35. ICAM-1 Knockout in Pluripotent Stem Cells Diminishes Immune Cell Adhesion

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Human pluripotent stem cells (PSCs) have ushered in a new era of regenerative medicine. Clinical translation of PSC-based therapies requires the prevention of immune allorejection following transplantation (tx). One promising strategy to address this barrier is hypoimmune gene editing to modify immune-associated genes. The first generation of hypoimmune PSC cell therapies includes knock-out (KO) of major histocompatibility complex genes to diminish T cell and antibody-mediated allorejection in PSC-derived cells. However, this strategy neglects innate immune cells (e.g., monocytes, neutrophils) which play key roles in acute allorejection.

Here, we interrogate a new hypoimmune gene target: ICAM-1, a cell adhesion molecule expressed by multiple PSC-derived cell types, including endothelial cells (ECs) and cardiomyocytes (CMs). ICAM-1 plays a critical role in the earliest steps of allorejection by binding directly to ligands expressed on all the major effector immune cells involved in allorejection. This binding is needed for formation of the immune synapse, an initial step to cytolysis, leading to post-tx immune cytotoxicity. It is also involved in key immune interactions with inflamed vasculature in transendothelial migration (TEM) during allorejection. During TEM, ECs lining blood vessel lumens upregulate ICAM-1 on their surface which allow the circulating leukocytes expressing LFA-1 and/or MAC-1 (ICAM-1 cognate ligands) to firmly adhere to the vasculature, extravasate into, and kill, the parenchymal cells of tx'd grafts.

Here, we demonstrate that blocking of surface ICAM-1 on wild type (WT) H9 PSC-derived CMs and ECs reduces leukocyte binding. We then show that KO of ICAM-1 in PSC-ECs significantly

reduces leukocyte binding compared to WT. Addition of ICAM-1 KO to the first-generation hypoimmune PSC cell therapies confers additional protection by generalized impedance of immune cell adhesion. Our results indicate that ICAM-1 KO could significantly benefit a wide range of multi-cellular, vascularized PSC-based therapies by disrupting the earliest initiation processes of allorejection.

36. Dissecting molecular mechanisms of YY1-mediated PcG regulation of hematopoietic stem cell metabolism

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Yin Yang 1 (YY1) is a multifunctional zinc-finger transcription factor and a polycomb group (PcG) protein that can recruit other PcG proteins to specific DNA sites. Notably, a small 26-amino acid YY1 REPO domain is necessary and sufficient for YY1's PcG functions. Our previous studies revealed that full length YY1 is critical for hematopoietic stem cell (HSC) long-term self-renewal and quiescence. YY1 REPO domain is essential for proper B and T cell development, however, it's function in HSC development and metabolism is largely unknown. This work focuses on understanding the intricate roles of YY1 REPO domain at HSC level which provides valuable insights into both normal development and disease pathogenesis. Conditional deletion of YY1 REPO domain was achieved by generating Yy1f/ΔREPO Mx1-Cre/ Vav-Cre mice, where the wildtype YY1 allele is deleted upon Cre recombinase expression, leaving the second allele with the germline REPO deletion. Compared with WT and YY1 heterozygote control mice, YY1 REPO domain deleted mice had expansion of immunophenotypic HSCs in bone marrow and the myeloid / lymphoid ratio in peripheral blood. However, the expanded HSCs fail to self-renew. Notably, Yy1-/ΔREPO mice had expansion of myeloid primed multipotent progenitors, increased myeloid specific colonies and increased CD61 surface expression on HSC indicating that REPO domain is critical for inhibiting myeloid-biased HSCs. Furthermore, Yy1-/ΔREPO HSCs showed decreased quiescence with increased proliferation, elevated ROS levels, and elevated mitochondrial membrane potential. Interestingly, all these phenotypes are hallmarks of aging related changes in hematopoiesis. Bulk RNA sequencing of Yy1-/ΔREPO HSCs revealed the genetic network governing HSC metabolism were deregulated. Our study supports that YY1 mediated PcG function/network is critical for HSC metabolism and inhibits HSC myeloid bias. Disruption of YY1 mediated PcG function leads to acceleration of HSC aging and dysfunctions. Our studies have elucidated innovative mechanisms and pathways by which epigenetic mechanisms dictate cell fate decisions during hematopoiesis.

37. A scalable 2D in vitro bone marrow niche model for high throughput screening of hematopoietic stem and progenitor cell mobilizing agents.

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Hematopoietic stem and progenitor cells (HSPCs) can replenish the entire blood population and reside within a tightly regulated niche of the bone marrow. HSPCs are regulated by a network of mesenchymal stromal cells (MSCs), endothelial cells (ECs), and other hematopoietic cells of the perivascular niche. Mobilized HSPCs are harvested for transplant as effective treatments for blood diseases and cancers. However, many HSPC donors do not respond well to the current

clinical mobilizer G-CSF and AMD3100, creating a critical need to find novel and improved mobilizing agents. We have developed an in vitro bone marrow niche platform that allows us to directly observe HSPC response to novel small molecules. To create the in vitro niche, a triple co-culture of fluorescently labeled blue HSPCs, green MSCs, and red ECs are assembled in a serum-free stem cell media. The 2D co-culture platform was scaled to 384-well optical plates and imaged on a microscopy- based plate reader for high throughput screening. An automated image analysis workflow was developed to quantify HSPC interactions with bone marrow niche cells. In measuring the distance of HSPCs to the closest MSC, our data shows that the majority of HSPCs in vitro remain near MSCs, consistent with the endogenous niche. To verify that the system accurately models endogenous behavior, we applied AMD3100 and found an increase in the distance between HSPCs and MSCs. Our in vitro high-throughput screening platform will be used to identify novel small molecules that can induce HSPC mobilization from the bone marrow.

38. Defining a Rejuvenation Signature in Transcription Factor Mediated Cellular Reprogramming

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In humans, advanced age is correlated with an increased incidence of debilitating and degenerative diseases. It is also correlated with cellular hallmarks such as the loss of the heterochromatin modification, H3K9me2, and an increase in the DNA damage marker, gH2AX. Remarkably, cellular hallmarks of aging are rejuvenated when somatic cells are reprogrammed into induced pluripotent stem cells (iPSCs) by the overexpression of the Yamanaka transcription factors – Oct4, Sox2, Klf4, and c-Myc (OSKM). Rejuvenation can also be achieved in reprogramming cells that are not yet iPSCs, by the acute expression of OSKM. Hence, we hypothesized that the mechanism of rejuvenation can be determined by a comparison of acute and continuous expression of OSKM. Thus, we carried out a reprogramming time course in OSKM, doxycycline (dox) inducible, aged, 16-month-old mouse fibroblast where we collected cells that are reprogramming (+Dox) or reverting back (-Dox) to their somatic cell identity in a rejuvenated state. The collected cells were subjected to single-nucleus (sn) multiome- (sn-RNA- and sn-ATAC-) sequencing to capture the gene expression and chromatin accessibility profiles. Tcfap2d and Wt1 motifs were identified to be enriched in the reverted and likely rejuvenated cell populations. Fibroblasts from mice ages 2-, 8-, and 24-month-old were also sequenced to serve as a landscape reference of young and old cells. Since each gene can be controlled by multiple transcription factors (TFs) and each TF can control multiple genes, this results in interactions that form a gene regulatory network (GRN). The snMultiomic data will be input for the construction of a rejuvenation-specific GRN with our advanced inference algorithm, scMTNI (single-cell Multi-task learning Inference). Nodes (TFs) from these GRNs will be validated for their role in rejuvenation by measuring changes in H3K9me2 and gH2AX as a proxy for cellular age, establishing a hierarchy of genes and their regulators that modulate rejuvenation.

39. Molecular Determinants of Innate Immune Signal-Dependent Genome Responsiveness in Hematopoietic Progenitor Cells

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GATA2 coding and noncoding germline mutations cause GATA2 deficiency syndrome, often leading to myelodysplastic syndrome and acute myeloid leukemia. GATA2 deficiency in murine fetal liver progenitors resulting from loss of Gata2 -77 enhancer upregulates components of Interferon-gamma (IFN⍰) and Toll-like receptor (TLR) signaling pathways. Using a genetic rescue system, we demonstrated that GATA2-deficient progenitors are hypersensitive to IFN⍰ and TLR1/2 agonist through signaling crosstalk to establish an aberrant transcriptional state. However, how hematopoietic regulators dictate genome responsiveness to a dynamically changing signaling environment in physiological and pathologic contexts is not fully established. GATA2 deficiency elevated expression of select B-lineage and myeloid genes via a PU.1-dependent mechanism. To test the hypothesis that PU.1 is required to establish an ectopic signaling state in GATA2-deficient progenitors, we used GATA2-deficient progenitors lacking Spi1 -14 kb distal enhancer (reducing PU.1 expression ~50%) and tested whether PU.1 is critical, contributory, or not required for IFN⍰- and TLR-dependent genomic responses. RNA-seq analysis revealed that PU.1 loss abrogated TLR1/2-dependent transcriptional regulation. By contrast, PU.1 loss attenuated certain, but not all, IFN⍰-mediated transcriptional responses or responses to synergistic signaling. Ongoing studies are assessing whether inflammation-induced signaling influences GATA2 and PU.1 chromatin occupancy and GATA2 deficiency impacts PU.1 occupancy. De novo motif enrichment analysis of RNA-seq data showed that genes activated by combinatorial signaling in GATA2-deficient cells harbored RUNX motifs. By contrast, RUNX motifs were absent in genes activated by combinatorial signaling in wildtype or GATA2-deficient, PU.1-deficient progenitors. Since RUNX1 is essential for HSPC generation and function, we asked if RUNX1 is required for genomic responses to signaling. RUNX1 deletion in GATA2-deficient progenitors attenuated the responsiveness of select IFN⍰- and TLR-activated genes (Ccl3, Ccl4, and Cxcl10) while not impacting others (Gbp2, Gbp7, and Cd40). In aggregate, these studies support a model in which GATA2 levels dictate genome responsiveness to the inflammatory signaling milieu, and GATA2 opposes the activity of PU.1 and RUNX1 to regulate signal-mediated transcriptional responses in context-dependent mechanisms.

40. Polycomb Group Protein Yin Yang 1 controls DNA Methylation in murine T Lymphocyte Development

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Transcription factors and epigenetic regulators play key functions in T lymphocyte lineage commitment and development. Mammalian Polycomb group (PcG) proteins are key negative regulators of gene expressions by binding to gene regulatory regions as large complexes. Many questions remain unanswered regarding how cell- and tissue-specificity is achieved by PcG proteins. While most PcG proteins lack DNA binding abilities, transcription factor Yin Yang 1 (YY1) can bind to DNA and recruit PcG proteins to DNA sites. YY1 REPO domain is necessary and sufficient for the recruitment of PcG proteins. While YY1ΔREPO is competent for DNA binding

and transcription regulations, YY1ΔREPO is defective in all YY1 PcG functions. Our prior study demonstrated that YY1 deletion in hematopoietic cells halts T cell development, and ectopic YY1ΔREPO expression fails to rescue T cell survival, emphasizing the importance of YY1 mediated PcG functions in T cell development. To explore this, we generated Yy1f/ΔREPO Vav-Cre (Yy1-/ΔREPO) mice by CRISPR/Cas9. Yy1-/ΔREPO mice exhibit reduced thymus size and cellularity with medulla aplasia, along with a developmental blockage at the DN3 stage. Yy1-/ΔREPO DN T cells show increased apoptosis and reduced TCRβ+ cells. Interestingly, RNA-seq analysis indicates dysregulated genetic networks governing DNA and histone methylation, chromosome maintenance, and translation initiation complex formation in Yy1-/ΔREPO mice. Additionally, DNA demethylases Tet1 and Tet2 are downregulated in Yy1-/ΔREPO DN3 thymocytes. Our data show that the YY1 PcG function is crucial for proper DNA methylation and T-cell development. As PcG proteins modify histone via trimethylation of histone H3 on lysine 27, we will further assess the impact of YY1 REPO domain/PcG function on histone methylation in T cell development. By utilizing a powerful mouse model for identifying YY1 functions as PcG domain-dependent or PcG domain-independent, our study has generated fundamental new insights into the epigenetic landscape in T cell development.

41. Single-cell trimodal profiling of transcript, cell surface proteins and chromatin accessibility in human erythropoiesis

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Erythropoiesis is a well characterized developmental process that involves the differentiation of hematopoietic stem/progenitor cells into red blood cells. Due to the unique features of being simple, robust, and well recapitulated in culture and in mice experiments, erythropoiesis is used as an ideal model system to address important biological questions such as how the transcription factors and cofactors work to dictate cell fate decisions or to maintain a cell identity.

Advances have been made at exploring different phases of erythropoiesis based on transcriptome and proteome landscape at bulk and single cell level. Single cell transcriptional studies suggested a continuous, hierarchical structure of hematopoiesis in both mice and human (Tusi et al., 2018; Pellin et al., 2019). Previous work in our group revealed protein abundance of lineage-specific transcription factors alter gradually, and notably, their quantitative changes can determine alternate cell fates in erythropoiesis (Palii et al., 2019).

However, the role played by chromatin structure remains unclear. Furthermore, single cell multi-omics studies to investigate erythropoiesis have remained limited. Here, using an approach named TEAseq, we performed single cell, simultaneous tri-modal measures of transcriptome (scRNAseq), chromatin (scATACseq) and 167 surface proteins at multiple time-points during differentiation from human hematopoietic stem/progenitor cells to terminally differentiated erythroid cells. We used integrated analysis on transcriptomic and epigenetic states in the same single cells to investigate the relative contributions of transcription factors and chromatin structure to the underlying regulatory mechanism driving cell fate choices in human erythropoiesis. Interestingly, we find a higher degree of discrepancy between chromatin structure and transcripts levels in progenitors compared to differentiated erythroid cells. Furthermore, besides the major erythroid lineage, a minor lineage of megakaryocyte was also

identified in our erythroid-promoting system, which confirms multipotency of hematopoietic progenitors in our differentiation system. Near the branch point, we observed changes in chromatin structure and transcripts levels, which suggests important contribution of epigenetic mechanisms in cell fate choice between megakaryocytes and erythroid cells. Our results provide new insights into the epigenetic regulation of erythropoiesis at the single cell level.

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42. Adult human physiologic media enhance structural maturation of human iPSCs-derived cardiomyocytes.

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While cardiomyocytes derived from human pluripotent stem cells (hPSC-CMs) have immense potential for cell therapy, disease modeling, and drug development, their immature phenotypes largely limit their utility for regenerative medicine applications. Attempts to mimic the metabolic environment during human fetal development by supplementing hPSC-CMs with physiologic glucose and oxidative substrates have shown promising results for improving cardiomyocyte maturation. Although these studies successfully pushed the sarcomere length in immature hPSC-CMs from 1.6 to 1.8μm, they did not achieve a mature sarcomere length of 2.2μm reflecting incomplete structural maturation. In this study, we applied the systematically developed human plasma-like medium (HPLM), which mimics physiologic substrate concentrations present in adult human plasma. We treated hPSC-CMs with HPLM for 2-weeks to assess the effects on structural, bioenergetic, and electromechanical maturation. hPSC-CMs treated with HPLM displayed increased sarcomere lengths ranging from 2-2.2μm and a transition to more mature myosin isoform expression from MLC-2a (immature) to MLC-2v (mature). Additionally, RNA sequencing indicated a metabolic shift from glycolysis to oxidative phosphorylation and improved calcium handling for the HPLM-treatment group. Consistently, HPLM-treated hPSC-CMs demonstrated higher basal respiration and maximal respiration by functional Seahorse analysis. In conclusion, by using HPLM, we improved the structural maturation of hPSC-CMs by achieving the maximum permissible sarcomere length and enhanced metabolic maturation through molecular and functional analysis.

43. Investigating the role of nascent transcription during pluripotency transitions

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During mouse embryogenesis, cells maintain pluripotency, representing their propensity to differentiate into all somatic and germline lineages. In vivo, pluripotent cells are present from embryonic days 3.5(E3.5) to E5.5 as pluripotent stem cells (PSCs) in vitro using specific signaling inhibitors and growth factors in the ground state, naïve state, formative state respectively. While this continuum of pluripotency has been characterized by different transcriptional and epigenetic traits, variance of de novo nascent RNA production between these states has been overlooked. Our preliminary data and previous publications found ground-state ESCs have the least nascent RNA production, followed by naïve-ESCs, and then formative-ESCs. Surprisingly, this range of nascent RNA production is inversely corelated with RNA polymerase II (RNAPII) abundance. Therefore, we hypothesize that the processivity of RNAPII, including its pause release and elongation rate, must be regulated. We have shown that a specific histone methyltransferase, DOT1L, impedes nascent RNA production during the conversion of somatic cells to induced pluripotency stem cells. Therefore, we have developed DOT1L loss-of-function and gain-of-function ESC models to further understand the regulatory role of nascent transcription during pluripotency transitions.